PMI VECTORLINK LIBERIA PROJECT

PMI VECTORLINK LIBERIA

ENTOMOLOGICAL MONITORING

FINAL REPORT DRAFT

OCTOBER 1, 2018–SEPTEMBER 30, 2019

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Abt Associates | 6130 Executive Blvd |
| Rockville, MD 20852 | T. 301.347.5000 | F. 301.913.9061
| www.abtassociates.com

CONTENTS

ACRONYMS

CDC	Centers for Disease Control and Prevention
CHW	Community health worker
ELISA	enzyme-linked immunosorbent assay
ITNs	insecticide-treated nets
LT	light trap
NMCP	National Malaria Control Program
PCR	**p**olymerase chain reaction
PMI	President's Malaria Initiative
PSC	pyrethrum spray collection

EXECUTIVE SUMMARY

VectorLink Liberia in collaboration with the country's National Malaria Control Program (NMCP) performed vector surveillance and insecticide resistance monitoring from October 2018 through September 2019. Adult mosquitoes were collected routinely (as part of longitudinal monitoring) from four sentinel monitoring sites: Fissebu (Lofa County), Madina (Grand Cape Mount County), Koryah (Bong County), and Saint John (Grand Bassa County). Two different methods of collection were used: pyrethrum spray collection (PSC), and Centers for Disease Control and Prevention (CDC) light traps (CDC-LTs). The main objective of these collections was to assess entomological parameters including vector composition, distribution, and behavior, and infection status of malaria vectors.

Insecticide resistance tests were performed in seven sentinel testing sites across the country to assess the susceptibility status of *Anopheles gambiae* s.l. to pyrethroids. Synergist tests were conducted with PBO to assess its impact on enhancing susceptibility of the vector to pyrethroids.

Using PSC and CDC-LT methods, a total of 2,329 *An. gambiae* s.l. and 354 *Anopheles funestus* s.l. were collected in the four monitoring sites (87% and 13%, respectively). *An. gambiae* s.l. was the predominant malaria vector in three out of the four, with percentages variable across the sites: Saint John (99%), Madina (99%), Koryah (77%), and Fissebu (43%). In Fissebu, the predominant vector was *An. funestus* s.l., at 54% of mosquitoes collected. Peak vector density was observed from April through June, which corresponds to the start of the rainy season, with density declining during the heaviest rains, which started in July and washed out many breeding areas. From the PSC, the highest indoor density of *An. gambiae* s.l. was recorded in Saint John site with 4.6 mosquitoes per house per day and the lowest in Fissebu at 0.35 per house per day. Collections from CDC-LT was generally low; with *An. gambiae* s.l. numbers ranging from 0.86 per trap per night in Saint John to 0.14 per trap per night in Madina.

Insecticide susceptibility tests using CDC bottle assay revealed that *An. gambiae* s.l. is resistant to deltamethrin at all sites with mortality rates to the diagnostic concentration (1x) ranging from 31 percent to 52 percent. Similarly, the vector was also resistant to permethrin with mortalities to 1x concentration ranging from 28 percent to 40 percent.

Pre-exposure to a synergist, piperonyl butoxide (PBO), increased susceptibility of *An. gambiae* s.l. to both deltamethrin and permethrin, but did not fully restore susceptibility. These findings suggest there may be other mechanisms of resistance to pyrethroids, which should be taken into consideration when selecting the best option for insecticide-treated net (ITN) distribution in Liberia.

1. INTRODUCTION

After indoor residual spraying in Liberia ended in 2014, PMI initiated routine entomological surveillance of malaria vectors in sentinel sites, and testing for insecticide resistance. Data collection on insecticide resistance among the malaria vectors is used to better understand the evolution of the resistance within the country, and will inform the selection of insecticides for use in ITNs.

Starting in October 2018, VectorLink began monthly, routine entomological monitoring in four sentinel sites - Madina, Fissebu, Koryah, and Saint John (Figure 1). These sites replaced three sites - Frank Town, Tomato Camp, and Jeneta - where routine collections had been done from 2016 through September 2018. PSCs and CDC-LT collections were used to assess the variation of vector density, composition, and behavior. The rational for the replacement was to generate data from new sentinel sites to increase vector surveillance coverage.

Insecticide resistance testing activities were conducted in an additional ten sites, where VectorLink assessed *An. gambiae* s.l. susceptibility to selected insecticides at different intensities, with synergist tests conducted on pyrethroids to test whether susceptibility could be restored following exposure to a synergist. VectorLink also tested *An. funestus* s.l. susceptibility to chlorefenapyr in one site.

FIGURE 1: VECTORLINK LIBERIA INSECTICIDE RESISTANCE AND ENTOMOLOGICAL MONITORING SITES, OCTOBER 2018–SEPTEMBER 2019

The main objectives of VectorLink Liberia entomological monitoring activities are as follows:

- Assess *Anopheles* vector bionomics, including species composition, density, and behavior, in four sites in four counties.
- Conduct molecular analyses to determine the sporozoite infection rates, blood-meal sources and identify species of vectors collected during routine collection as well as identify species and potential mutations contributing to insecticide resistance for samples undergoing insecticide susceptibility testing.
- Determine insecticide susceptibility of *An. gambiae* s.l., the primary local malaria vector, to pyrethroids in seven sites in seven counties.
- Determine insecticide susceptibility of *An. gambiae* s.l. to chlorfenapyr in six sites across six counties.
- Determine insecticide susceptibility of *An. funestus* s.l. in sites where found to be dominant vector.
- Maintain and support a functional insectary.
- Build local capacity in entomological surveillance methods and techniques through formal and informal training.

2. MATERIALS AND METHODS

2.1 DESCRIPTION OF SAMPLING SITES FOR ROUTINE ENTOMOLOGICAL MONITORING (VECTOR BIONOMICS)

VectorLink Liberia provided support to the NMCP to enable it to conduct monthly vector bionomics monitoring in four sites and bi-annual insecticide resistance testing in 15 counties, alternating between 7-8 counties each year to enable greater geographic coverage (Table 1). Sites were chosen in collaboration with the NMCP to provide a wide geographic coverage across Liberia. Specifically, sites were to be accessible, in close proximity to breeding sites, and have at least 20 households.

TABLE 1: SUMMARY OF SITES FOR ENTOMOLOGICAL SURVEILLANCE AND INSECTICIDE RESISTANCE MONITORING ACTIVITIES

Region	County	Insecticide Resistance Monitoring Site	Vector Bionomics Monitoring Site
North Central	Nimba	Zolowee	
		Gbedin**	
	Lofa	Zorzor*	Fissebu
	Bong	CARI**	Koyah
South Central	Grand Bassa		Saint John
	Margibi	Jackson Farm**	
North Western	Gbarpolu	Bopolu	
	Grand Cape Mount	Sinjeh	Madina
South Eastern A	Grand Gedeh	Zwedru	
	Sinoe	Greenville	
South Eastern B	Grand Kru	Barclayville	

*Zorzor replaced Fissebu as an insecticide resistance monitoring site due to inability to locate sufficient larvae in Fissebu.
**Only chlorefenapyr was tested in these sites.

For insecticide resistance monitoring using pyrethroid, a sentinel site was selected in seven out of the 15 counties in Liberia so that data is gathered from each county at least every other year to inform ITN selection, with specific sites sometimes chosen to ensure representation of different vector species and typical malaria vector breeding areas (e.g., rice fields and swamps) (Annex A). Chlorfenapyr was tested in six sites including an additional three sites which were not originally targeted. These sites were selected as they allowed for safe transport of larvae to the insectary, where electricity was relatively stable to support the three day observation period required for chlorfenapyr testing. In total, insecticide test was done in 10 sites across the country.

2.1.1 ADULT MOSQUITO COLLECTIONS

Two methods were used to collect adult mosquitoes in the four routine monitoring sites: PSC and CDC-LTs. See Table 2 for a summary of the methods. Human landing catches (HLC) were not conducted, because in previous years the number of mosquitoes collected using this method was low due to the rain. The CDC-LTs were used instead, as a proxy method to HLC, by counting mosquitoes collected every two hours.

TABLE 2: ADULT MOSQUITO COLLECTION METHODS USED FOR LONGITUDINAL MONITORING

Collection method	Time	Frequency	Sample
PSC	6:00 a.m. to 8:00 a.m.	Monthly	20 houses per site (10 houses each day for two days)
CDC-LT	6:00 p.m. to 6:30 a.m.	Monthly	Eight houses per site (four houses per night for two nights), using eight CDC-LTs—four indoors, four outdoors

For PSC, white sheets or other large pieces of white cloth were placed on the floor from wall to wall in sampled houses. The teams sprayed the commercial pyrethroid insecticide, Kwik,[1] in the house after closing windows, doors, and any other open spaces around the house. All food and drinking water were covered or removed from the house before spraying. After a 10-minute knockdown period, the sheets were collected.

CDC-LTs were set-up both outdoors and indoors at selected houses where people slept under a mosquito net. The head of each participating household consented beforehand. One indoor and one outdoor trap were used per participating household. The indoor traps were placed approximately 150 cm above the sleeper's legs. The outdoor CDC-LTs were set up at least four meters from the same houses that contained the indoor traps, and were baited with carbon dioxide (sourced using yeast + sugar + water). The following indicators were calculated based on the number of mosquitoes collected through each collection method:

Collection Method	Indicator	Definition
PSC	Indoor Resting Density	# mosquitoes/house/day
CDC-LT	Indoor Density	# mosquitoes collected indoors/trap/night # mosquitoes collected indoors/trap/2 hours
	Outdoor Density	# mosquitoes collected outdoors/trap/night # mosquitoes collected indoors/trap/2 hours

All mosquitoes collected through each method were identified morphologically under a dissection microscope, in the field, by the team of highly trained technicians from the University of Liberia, the NMCP, and VectorLink. The *An. gambiae* s.l. mosquitoes were counted and sorted based on their blood-digestion stage. All *Anopheles* species identified (Gillies and Coetzee 1987) were preserved on silica gel for further sample processing in the laboratory at LIBR using enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) techniques.

[1] Kwik ingredients: Transfluthrin 0.05% ; Tetramethrin 0.20% ; B-Cyfluthrin 0.20% ; PBO 0.50% ; Solvent & Propellant 99.55, ASI Al Sharhan Industries, Kuwait

2.2 INSECTICIDE RESISTANCE MONITORING

Insecticide resistance monitoring tests were done across ten sites in ten counties (Table 3). In most cases, VectorLink, the NMCP, and CHWs collected the necessary larvae, reared them to adulthood, and conducted testing in the field. Sometimes in an effort to save time and/or costs, larvae were transported to the container box insectary in Monrovia for rearing and to perform assays on the adult female *Anopheles* malaria vector.

Per the work plan, the sentinel site in Lofa County was indicated as Fissebu, but that site was later replaced by the nearest town, Zorzor, due to insufficient *An. gambiae* s.l. larvae found in the original site. Because the distance between these sites is only five km, their vector populations were assumed to be similar.

At Zolowee, in Nimba County, mouth aspirators and Prokopack aspirators were used to collect adult *An. funestus* s.l. in order to recover eggs for rearing of F1 progeny adults. All *Anopheles* mosquitoes were identified alive in a mouth aspirator under a dissecting microscope to select the blood-fed *An. funestus* s.l. females for eggs-hatching. The rearing was completed in the insectary, because this species can take up to 20 days to progress from egg to adult.

TABLE 3: SUMMARY OF INSECTICIDE SUSCEPTIBILITY TESTS

Insecticide	Test*	Method	Sites
Deltamethrin	• Susceptibility and resistance intensity testing (1X, 2X, 5X, and 10X diagnostic dose) • Synergist assay	CDC bottle assay	Bopolu/Gbarpolu; Zorzor/Lofa; Sinjeh/Grand Cape Mount; Greenville/Sinoe; Barclayville/Grand Kru; Zwedru/Grand Gedeh; Zolowee/Nimba
Permethrin	• Susceptibility and resistance intensity testing (1X, 2X, 5X, and 10X diagnostic dose) • Synergist assay	CDC bottle assay	Barclayville/Grand Kru; Zwedru/Grand Gedeh; Zolowee/ Nimba
Chlorfenapyr**	Susceptibility tests using chlorfenapyr 100µg/bottle as interim diagnostic concentration	CDC bottle assay	CARI/Bong, Jackson Farm/Margibi, Zolowee/Nimba, Gbedin/Nimba, Zwedru/Grand Gedeh and Sinjeh/Grand Cape Mount.

**Deltamethrin took priority over permethrin if not enough larvae was collected to do both for diagnostic dose, intensity assays, and synergist assays.*

***Chlorfenapyr was only done where larvae could be moved to the insectary, where power was relatively stable, allowing for three days of observation.*

For each test, the team preserved a subsample of dead mosquitoes as well as all the survivors, which were stored in labeled Eppendorf tubes for further laboratory analysis.

2.2.1 INSECTICIDE RESISTANCE TESTS FOR CHLORFENAPYR USING THE CDC BOTTLE ASSAY METHOD

Chlorfenapyr was diluted in acetone to reach a concentration of 100µg/bottle. Four bottles were coated with the solution, and a fifth bottle coated with acetone-only for use as a control. Twenty, two- to five-day-old female *An. gambiae* s.l. mosquitoes were transferred into each of the exposure and control bottles (total 100 mosquitoes per test used). The number of knockdowns was recorded every 15 minutes for

two hours. After one hour of exposure, mosquitoes were transferred to cups and fed with a 10% sugar solution. Mortality was recorded every 24 hours for three consecutive days.

2.2.2 Insecticide Resistance Tests and resistance Intensity Assays for Pyrethroids Using the CDC bottle assay Method

For the susceptibility and resistance intensity assays for the pyrethroids tested (deltamethrin and permethrin), CDC provided the vials containing the technical grade insecticide concentrations. The diagnostic doses 1X (12.5 µg/bottle), 2X (25 µg/ bottle), 5X (62.5 µg/bottle), and 10X (125 µg/bottle) of each insecticide were tested at each site.

The dilution was done with 50 ml acetone per tube. Four bottles were coated with one ml of different concentrations of the prepared insecticide solutions. The control bottles were coated with one ml acetone only. All the bottles were kept overnight to dry before use the next day. Twenty-five female *An. gambiae* s.l. two to five days old were transferred into each of the tubes, for a total of 125 mosquitoes tested. The mortality was recorded every 15 minutes up to two hours.

2.2.3 Synergist Assay Using the CDC Bottle Method

Pre-exposure to a synergist (PBO) was done before introducing mosquitoes to the diagnostic doses of deltamethrin and permethrin to see whether susceptibility could be restored. A concentration (100 µg/bottle) of PBO was diluted with 50 ml acetone solution. One bottle was coated with one ml of PBO solution to be used as the synergist-exposure bottle. A second bottle was coated with one ml of acetone to serve as a synergist-control bottle. These were left to dry overnight. A subsample of 125 female *An. gambiae* s.l. was introduced into the synergist-exposure bottle for one hour. Another 125 female *An. gambiae* s.l. were introduced for one hour into the synergist-control bottle coated with acetone only.

After one hour, the mosquitoes were transferred to two holding cages—one for the synergist-control mosquitoes and another for the synergist-exposure mosquitoes. Four replicate tests were done for PBO and non-PBO, based on the CDC bioassays method, using eight bottles (four bottles for PBO and four bottles for non-PBO). In each insecticide-coated and control bottle, 25 females were introduced using a mouth aspirator, and mortality was recorded every 15 minutes, up to two hours. When control mortality was higher than 5% and less than 20%, mortality data were corrected with Abbott's formula – greater than 20% mortality among control mosquitoes, the tests were discarded. With more than 20 percent mortality among control mosquitoes, the tests were discarded.

2.3 Laboratory Analyses

The mosquito sample processing started for the first time in Liberia in April 2018. However, the sample analysis was suspended due to technical issues with the ELISA reader. VectorLink purchased a new reader, which was delivered in October 2019. The work will resume as soon as the new work plan for 2019–2020 is approved and the subcontract with National Public Health Institute of Liberia (NPHIL) is signed. Once the backlog for ELISA analysis is gone, VectorLink will work with LIBR to train their staff on PCR techniques for the molecular identification of *An. gambiae* s.l. complex and *An. funestus* s.l. Also, PCR will be conducted to identify mutations involved in target-site resistance mechanisms, including knockdown resistance and *Ace-1*. Once analyses are completed, results will be presented in the next report.

3. RESULTS AND DISCUSSION

3.1 ROUTINE ENTOMOLOGICAL COLLECTIONS

In total, 4,057 mosquitoes were collected in the four routine monitoring sites: 57% *An. gambiae* s.l., 9% *An. funestus* s.l., and 32% *Culex*. Among the 2,329 *An. gambiae* s.l. collected across the four sites, 83% were caught by PSC and 17% with CDC-LTs (Table 4). *An. gambiae* s.l. was the dominant vector in three of the four sites (Koryah, Saint John, and Madina), with *An. funestus* s.l. being the dominant vector in Fissebu (Figure 2).

The number of *An. funestus* s.l. (n =170) collected in Fissebu was significantly higher than *An. gambiae* s.l. (n=133; p=0.03). Significant numbers of *An. funestus* s.l. (n=178) were also found in Koryah (p<0.05). These two sites have more suitable breeding areas for *An. funestus* s.l. as compared with the two other sites, where the numbers of *An. funestus* s.l. were very low.

FIGURE 2: SPECIES COMPOSITION OF *ANOPHELES* MOSQUITOES COLLECTED BY PSC AND CDC-LTS FROM FOUR SENTINEL SITES, OCTOBER 2018–SEPTEMBER 2019

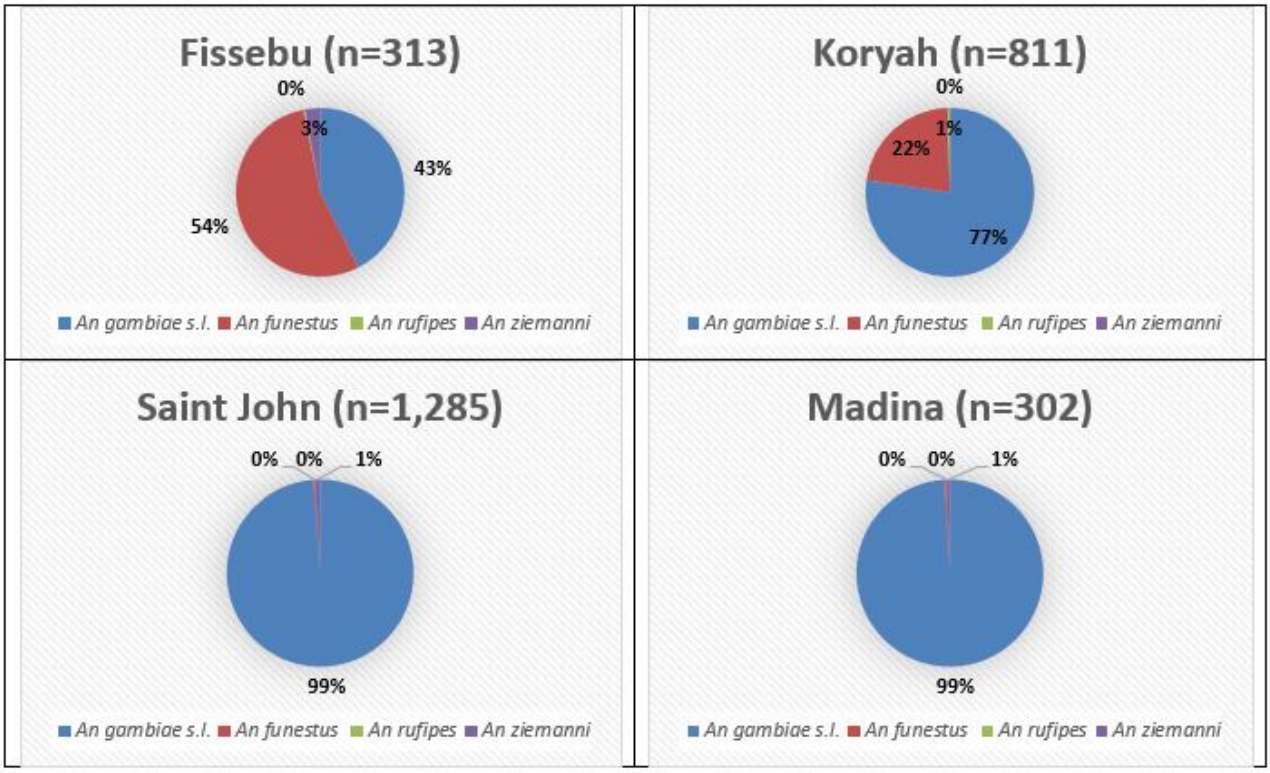


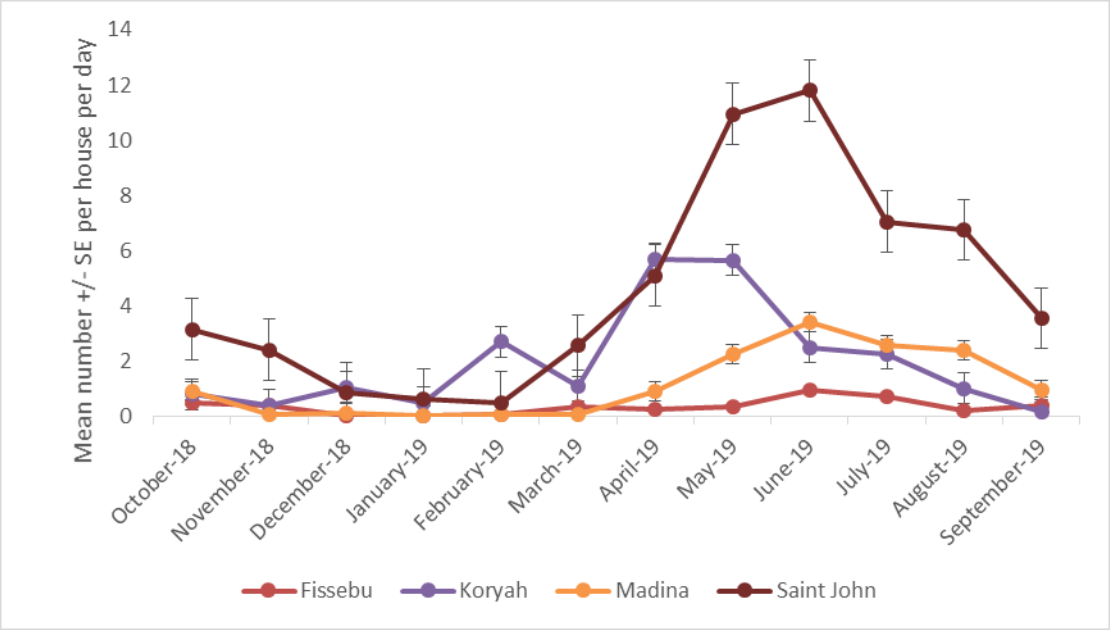
TABLE 4: *ANOPHELES* AND OTHER MOSQUITOES SPECIES COLLECTED IN THE FOUR ROUNTINE MONITORING SENTINEL SITES, OCTOBER 2018–SEPTEMBER 2019

Site	Method	*An. gambiae* s.l.	*An. funestus* s.l.	*An. rufipes*	*An. ziemanni*	*Culex*	*Aedes*	*Mansonia*
Fissebu	PSC	83	123	0	0	9	0	0
	CDC-LT Indoor	31	28	1	3	94	1	4
	CDC-LT Outdoor	19	19	0	6	156	1	5
	Total 1	*133*	*170*	*1*	*9*	*259*	*2*	*9*
Koryah	PSC	476	128	0	0	14	0	0
	CDC-LT Indoor	102	30	3	0	113	1	10
	CDC-LT Outdoor	49	22	1	0	172	0	21
	Total 2	*627*	*180*	*4*	*0*	*299*	*1*	*31*
Madina	PSC	272	1	0	0	41	2	0
	CDC-LT Indoor	9	0	0	1	308	0	2
	CDC-LT Outdoor	18	0	0	1	295	0	0
	Total 3	*299*	*1*	*0*	*2*	*644*	*2*	*2*
Saint John	PSC	1,105	0	0	0	11	0	0
	CDC-LT Indoor	98	2	0	4	27	4	0
	CDC-LT Outdoor	67	1	0	8	54	1	0
	Total 4	*1,270*	*3*	*0*	*12*	*92*	*5*	*0*
Total		**2,329**	**354**	**5**	**23**	**1,294**	**10**	**42**

3.1.1 INDOOR RESTING DENSITY

Overall 1,936 female *An. gambiae* s.l. were collected using the PSC method in Saint John (57%), Koryah (25%), Madina (14%), and Fissebu (4%). The highest indoor resting density was observed in June in Saint John (11.8 mosquitoes/house). Across all sites, the peak vector indoor resting densities were observed from April through June (Figure 3). Densities decreased from July through September, which is the peak rainy season when the heavy rains wash out the breeding areas.

FIGURE 3: MEAN MONTHLY INDOOR RESTING DENSITIES OF *AN. GAMBIAE* S.L. AS COLLECTED BY PSC, IN FOUR SITES, OCTOBER 2018–SEPTEMBER 2019



The majority of *An. gambiae* s.l. collected by PSC were blood-fed in all four vector bionomics monitoring sites (Figures 4–7).

FIGURE 4: ANNUAL DISTRIBUTION OF *AN. GAMBIAE* S.L. MOSQUITOES BY ABDOMINAL STAGE, COLLECTED BY PSC IN SAINT JOHN, OCTOBER 2018–SEPTEMBER 2019

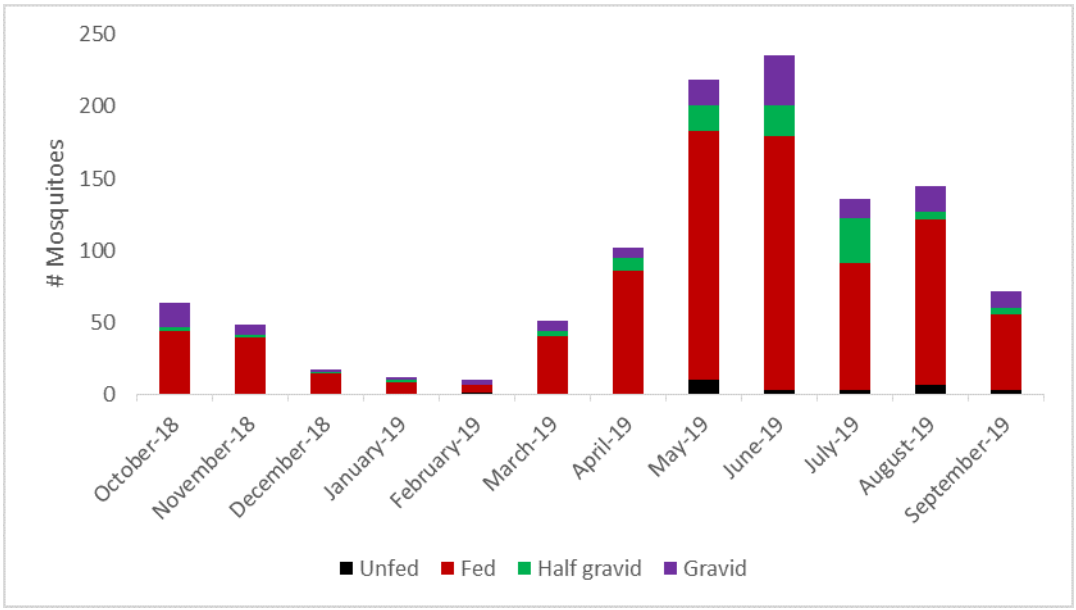


FIGURE 5: ANNUAL DISTRIBUTION OF *AN. GAMBIAE* S.L. MOSQUITOES BY ABDOMINAL STAGE, COLLECTED BY PSC IN KORYAH, OCTOBER 2018–SEPTEMBER 2019

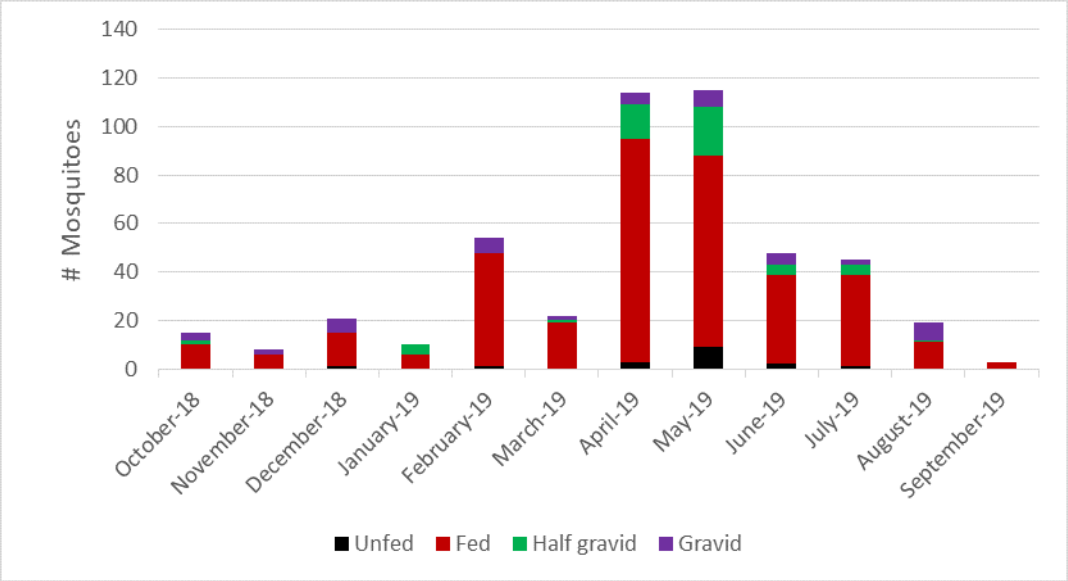


FIGURE 6: ANNUAL DISTRIBUTION OF *AN. GAMBIAE* S.L. MOSQUITOES BY ABDOMINAL STAGE, COLLECTED BY PSC IN FISSEBU, OCTOBER 2018–SEPTEMBER 2019

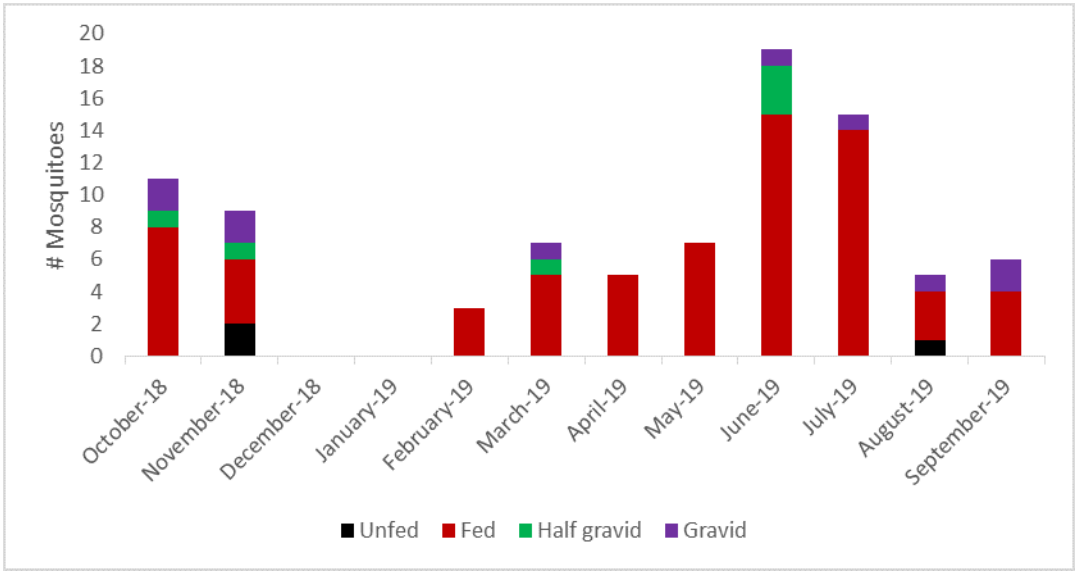
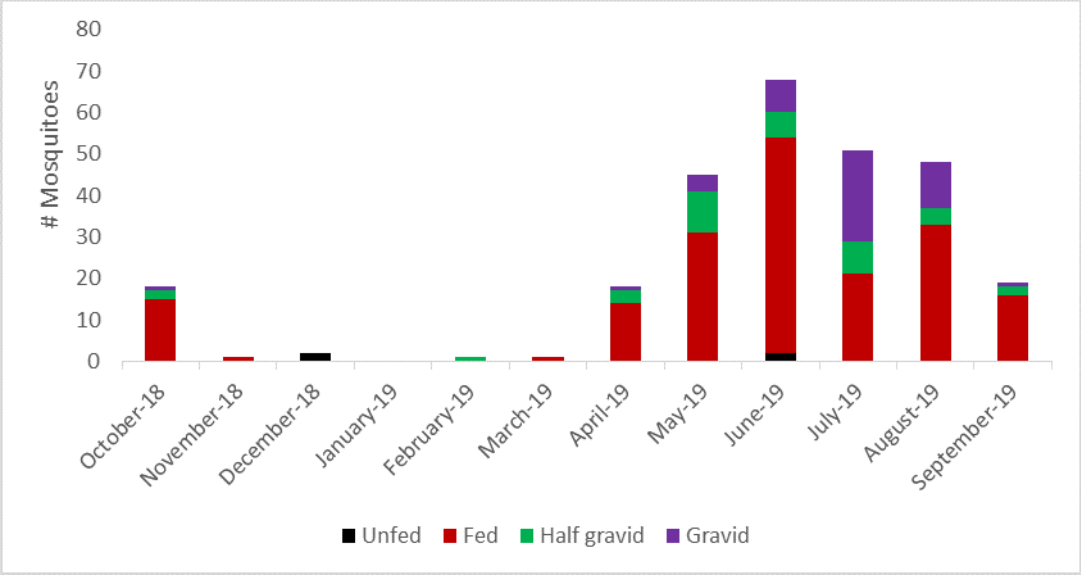


FIGURE 7: ANNUAL DISTRIBUTION OF *AN. GAMBIAE* S.L. MOSQUITOES BY ABDOMINAL STAGE, COLLECTED BY PSC IN MADINA, OCTOBER 2018–SEPTEMBER 2019



3.1.2 CDC-LT COLLECTIONS

From October 2018 through September 2019, 393 *An. gambiae* s.l. were collected using CDC-LTs in the four sites. Of these, 61% were collected indoors (Table 5). The highest numbers caught indoors for *An. gambiae* s.l. were recorded from April through June in three of the four sites (Annex B to E).

In Saint John, Fissebu, and Koryah the number of mosquitoes caught indoors tended to increase during the second half of the night (Table 5). The density per outdoor trap also increased in the second half of the night (Table 6). In Madina, the numbers of mosquitoes collected were too low to make any meaningful conclusions. Similarly, insufficient numbers of *An. funestus* s.l. were collected using CDC-LTs to make any meaningful conclusions.

TABLE 5: NUMBER OF AN. GAMBIAE S.L. COLLECTED INDOORS EVERY TWO HOURS PER TRAP BY CDC-LT, OCTOBER 2018–SEPTEMBER 2019

Time	Fissebu	Koryah	Madina	Saint John	Total
06-08 PM	3	27	2	21	53
08-10PM	5	14	3	10	32
10-12AM	2	8	1	0	11
12-02 AM	1	26	1	8	36
02-04 AM	12	6	0	15	33
04-06AM	8	21	2	44	75
Grand Total	**31**	**102**	**9**	**98**	**240**

TABLE 6: NUMBER OF *AN. GAMBIAE* S.L. COLLECTED OUTDOORS EVERY TWO HOURS PER TRAP BY CDC-LT, OCTOBER 2018–SEPTEMBER 2019

Time	Fissebu	Koryah	Madina	Saint John	Total
06-08 PM	1	12	7	5	25
08-10PM	1	0	4	6	11
10-12AM	1	8	1	0	10
12-02 AM	1	7	2	4	14
02-04 AM	4	4	2	8	18
04-06AM	11	18	2	44	75
Grand Total	**19**	**49**	**18**	**67**	**153**

For *An. gambiae* s.l. in Koryah and Saint John, significantly more mosquitoes were collected indoors than outdoors (Table 7). However, this may not explain the true preference of the mosquito as the bait indoors was different from the bait outdoors. There is no significant difference between the numbers of *An. funestus* s.l. collected indoors versus outdoors in either of the sites where a large number of the vector was found.

TABLE 7: DISTRIBUTION OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. MOSQUITOES COLLECTED INDOORS AND OUTDOORS IN THE FOUR ROUTINE, SENTINEL SITES UNSING CDC-LTS, OCTOBER 2018–SEPTEMBER 2019

	***An. gambiae* s.l.**				***An. funestus* s.l.**			
Site	**Indoor**	**Outdoor**	**Total**		**Indoor**	**Outdoor**	**Total**	
Fissebu	31	19	50		28	19	47	
Koryah	102	49	151		30	22	52	
Madina	9	18	27		0	0	0	
Saint John	98	67	165		2	1	3	
Total	**240**	**153**	**393**		**60**	**42**	**102**	

The outdoor traps were baited with CO2. However, we had no device in the field that could monitor the volume of CO2 released from the gallon throughout the night. The yeast and sugar likely did not provide sufficient CO2 to attract mosquitoes. Because of these technical variabilities, we could not relate the number of mosquitoes caught outdoors with feeding behavior. Next year, VectorLink will replace this proxy measure with human landing catches.

3.2 Insecticide Resistance Monitoring

3.2.1 Insecticide Resistance Testing of Pyrethroids at Diagnostic Dosages and Intensity Assays

In the seven insecticide resistance monitoring sites, *An. gambiae* s.l. mosquitoes were highly resistant to deltamethrin, with resistance found at all doses (1X, 2X, 5X, and 10X the diagnostic dose) after 30 minutes of exposure (Figure 8). The mortality rate for deltamethrin at 10X the diagnostic dose was 96%, which is below 98% susceptibility threshold which is indicated as a dotted line in the two figures below. Similarly, mosquitoes were resistant to permethrin at the four concentrations (1X, 2X, 5X, 10X) in the three sites where sufficient mosquitoes were available for tests (Figure 11).

FIGURE 8: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. FROM DIFFERENT SITES IN LIBERIA EXPOSED TO 1X, 2X, 5X, AND 10X DELTAMETHRIN DIAGNOSTIC DOSE USING CDC BOTTLE ASSAYS, NOVEMBER 2018–SEPTEMBER 2019

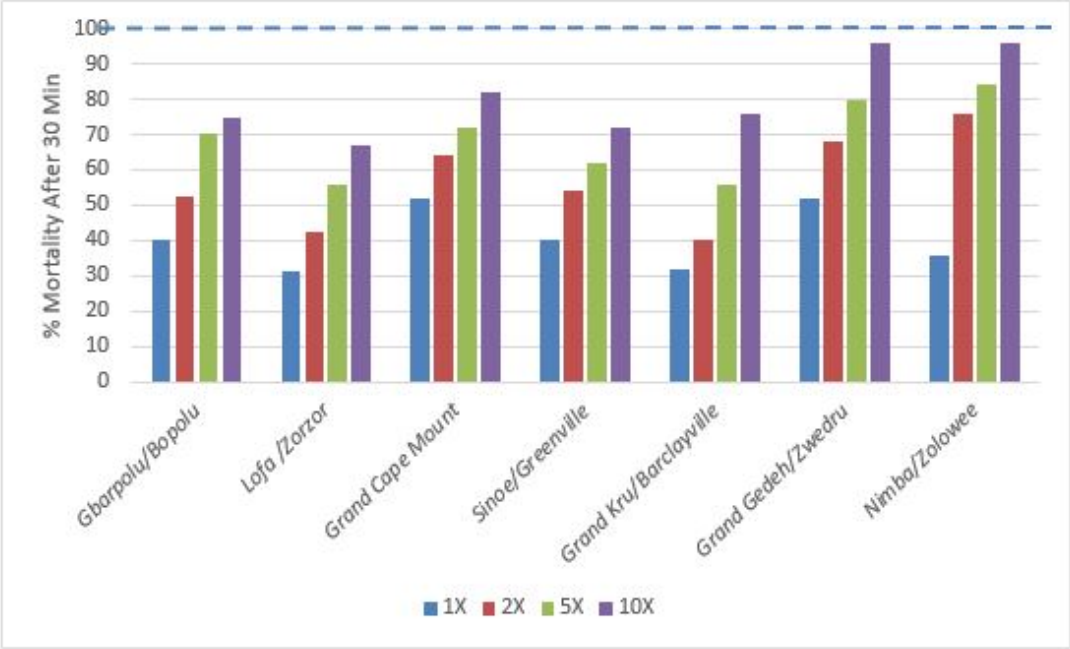
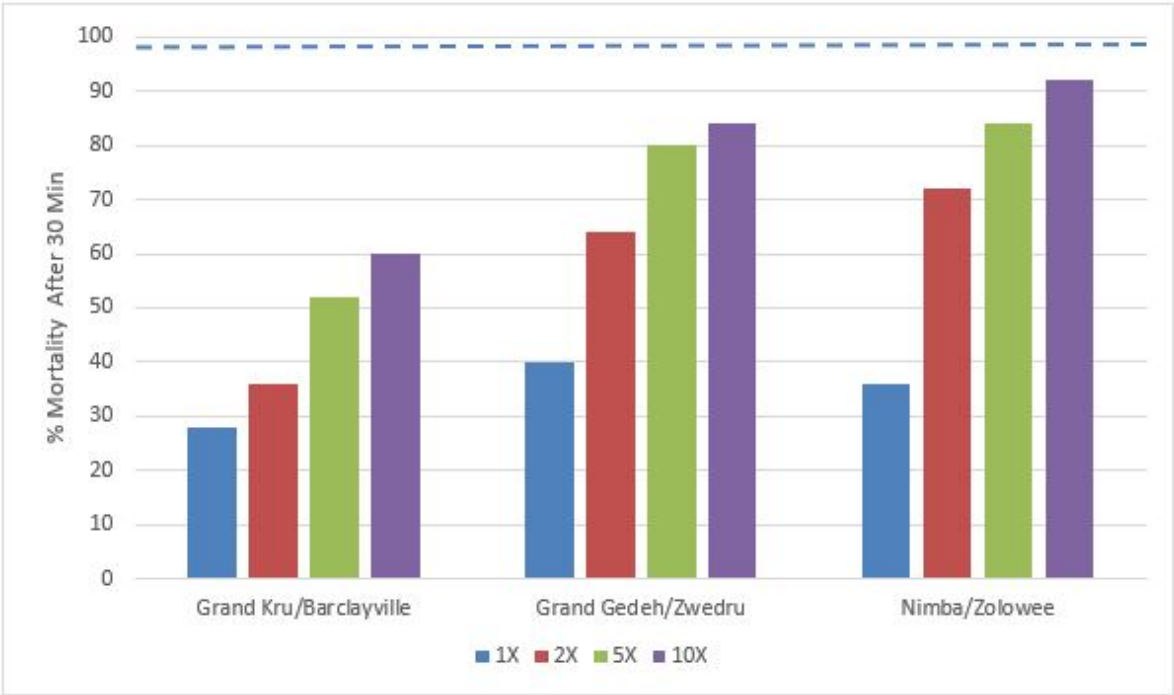


FIGURE 9: MORTALITY RATE OF *AN. GAMBIAE* S.L., PER EXPOSURE DOSE TO 1X, 2X, 5X, AND 10X OF PERMETHRIN DIAGNOSTIC DOSE, USING CDC BOTTLE ASSAYS, IN THREE COUNTIES, JUNE THROUGH AUGUST 2019



3.2.2 SYNERGISTS ASSAYS WITH PYRETHROIDS USING THE CDC BOTTLE ASSAY

For sites where synergist assays with deltamethrin and permethrin were conducted, there was a marked increase in mortality rates of *An. gambiae* s.l. after exposure to PBO; however, PBO did not restore full susceptibility at the 30-minute diagnostic time (Figure 10). Similar results were found when exposing permethrin to PBO, with the exception that full susceptibility was restored in one site – Zolowee in Nimba County (Figure 11). These data and the previous-year's results suggest the existence of mechanisms of resistance other than oxidases. Future molecular lab data analysis will work to identify possible mutations involved in the target site resistance.

FIGURE 10: MORTALITY RATE OF *AN. GAMBIAE* S.L. PER EXPOSURE TO DELTAMETHRIN AND DELTAMETHRIN PLUS PBO USING CDC BOTTLE ASSAYS, IN SIX COUNTIES, NOVEMBER 2018–SEPTEMBER 2019

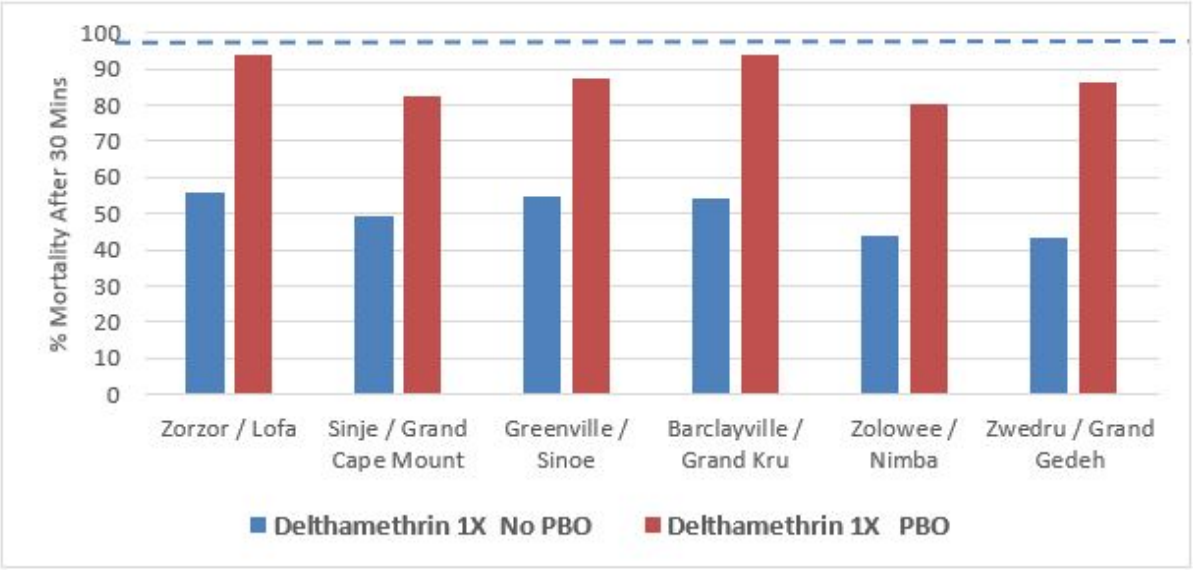


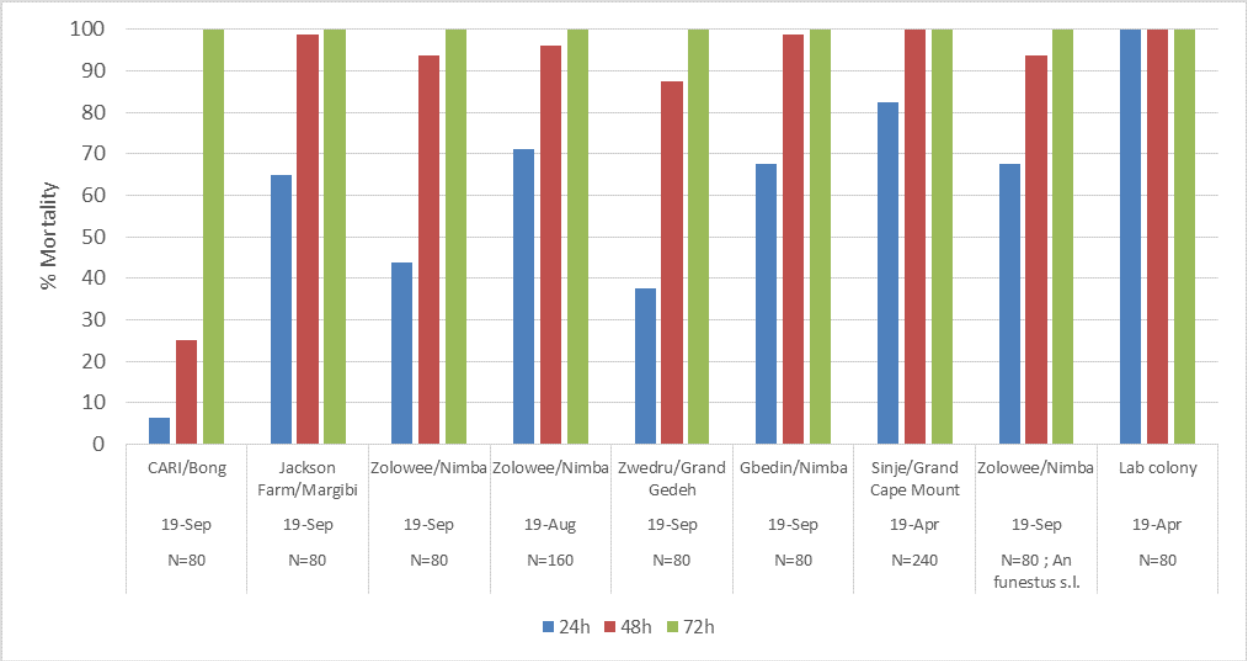
FIGURE 11: MORTALITY RATE OF *AN. GAMBIAE* S.L. PER EXPOSURE TO PERMETHRIN AND PERMETHRIN PLUS PBO USING CDC BOTTLE ASSAYS, IN THREE COUNTIES, JUNE- AUGUST 2019

3.2.3 INSECTICIDE RESISTANCE TESTING OF CHLORFENAPYR

Twenty-four hours post-exposure to chlorfenapyr, mosquito mortality rates were below 85% across all sites (Figure 12). After 48 hours, mortality rates increased with one site reaching 100% mortality – Sinje in Grand Cape Mount. After 72 hours, mortality reached 100% in all the sites. Given the number of *An. funestus* s.l. found in Zolowee, VectorLink conducted susceptibility testing using chlorfenapyr on this vector. In addition, one test was done using the lab colony *An. gambiae* Kisumu strain, which was fully susceptible after 24 hours.

Overall, both species sampled were susceptible to chlorfenapyr, at all sites. These preliminary data suggested that chlorfenapyr could be an alternative insecticide used to mitigate the high resistance observed with the pyrethroids.

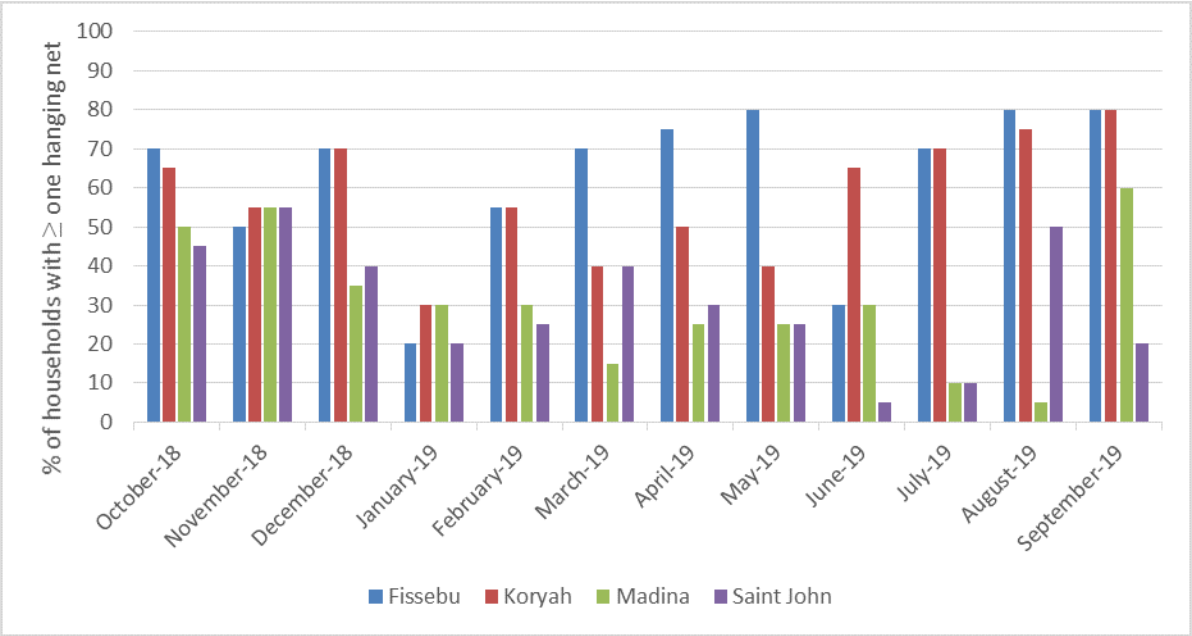
FIGURE 12: MORTALITY RATE OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. PER EXPOSURE TO CHLORFENAPYR USING CDC BOTTLE ASSAY.



3.3 PRESENCE OF HANGED NETS

The presence of hanged nets was monitored monthly in households as part of each PSC collection. Presence was defined as at least one hanging net in the home at the time of PSC collection. Only hanging (not stored) nets were recorded, since these are expected to be used by the sleeper. PSC was conducted in 20 houses, per site, per month. In Fissebu and Koryah, the proportion of hanged nets were generally higher than in the two other sites (Figure 13).

FIGURE 13: PROPORTION OF HOUSEHOLDS WITH HANGING NET AT THE TIME OF PSC FROM OCTOBER 2018 -- SEPTEMBER 2019



3.4 CAPACITY BUILDING ACTIVITIES

During this reporting period, VectorLink worked to improve local capacity in entomological monitoring (Table 8). VectorLink provided training on vector monitoring and insecticide resistance assays to five NMCP staff and one University of Liberia staff, who supervised CHWs at sentinel sites. At each sentinel site, four CHWs were trained on basic morphology of adult and larval mosquitoes. Where insecticide resistance tested was performed, CHWs received an introductory training on insecticide resistance testing using the CDC bottle method.

In Nimba County, VectorLink captured live *Anopheles* using a mouth aspirator and Prokopack, identifying the mosquitoes morphologically to select the female *An. funestus* s.l. for eggs-hatching in the insectary for use in insecticide resistance testing. VectorLink trained NMCP staff on containment measures to prevent mixing of this species with the lab colony strain.

TABLE 8: SUMMARY OF TRAININGS

Training	NMCP	CHWs	University of Liberia	Total
Insecticide resistance testing	4	28	0	32
Adult mosquito collection methods refresher trainings	0	16	1	17
Field morphological ID	0	44	1	45

VectorLink is currently working with LIBR and NMCP to relocate the insectary from the NMCP to the LIBR space in Charleville, as power in the current location is unstable, putting sustainability of the mosquito colony at risk.

4. OBSERVATIONS AND CONCLUSIONS

In the four vector bionomics monitoring sites, the vector abundance was high from April through June, and declined from July through September, which is consistent with previous years' observations. This finding confirmed that the peak of mosquito population coincided with the start of the rainy season, while the decline coincided with the heaviest rains (July through September), which wash out mosquito breeding areas. For vector control planning, that period of abundance should be considered for vector control interventions to have the greatest impact on the populations of *An. gambiae* s.l. and *An. funestus* s.l., the two main malaria vectors in Liberia.

In the new vector bionomics monitoring sites, the numbers of mosquitoes collected were relatively low. Carefully planned and supervised HLC collections could be a good option to confirm whether biting rates are also really low as indicated by the PSC or CDC-LT collections. However, even with lower abundance, Liberia continues to face a high-burden of malaria which could be the result of insufficient access to or use of protection measures and treatment.

The majority of *An. gambiae* s.l. collected through PSC were either fed, half-gravid or gravid *An. gambiae* s.l., which indicates there is still a significant amount of contact between hosts and vectors, despite the use of ITNs. This contact could increase the risk of exposure to malaria infection. However, it is yet to be confirmed whether these hosts were human, which will be done once fed samples are processed through ELISA for blood meal sources.

The numbers of mosquitoes collected in all sites using CDC-LT were low compared with the numbers collected using the PSC method. In two of the four sentinel sites (Fissebu and Koryah), *An. funestus* s.l. was the predominant. Processing the mosquito samples for *Plasmodium* infection will allow assessment of this species' contribution to malaria transmission in the two sites.

More mosquitoes were collected indoors than outdoors using the CDC-LTs. This could due to the difference in bait; human with indoor traps and CO2 produced from yeast and sugar with outdoor traps. The use of CO2 sources (yeast and sugar solution) for outdoor traps did not increase the number of mosquitoes collected. For host-seeking assessment, dry CO2 is preferable for outdoor trap-baiting, but it is not available in country. In the next work plan year, the team will use CDC-LTs for indoor mosquito collections only, and will resume collections outdoors with HLC to better determine biting behavior, following discussions with the NMCP.

The bioassays performed using the CDC bottle method confirmed that *An. gambiae* s.l. populations were highly resistant to pyrethroids. There was a marked increase in susceptibility to both deltamethrin and permethrin with pre-exposure to PBO; however, the use of the synergist did not fully restore susceptibility in most of the sites. Since the synergist test data show that mechanisms other than monooxygenase enzymes could be involved in the resistance observed, the target site resistance mechanism needs investigating. This will be done as part of laboratory testing.

For the first time, based on the insecticide resistance monitoring data generated by VectorLink and the NMCP, the country has decided to distribute Interceptor G2 nets (combining alpha-cypermethrin and chlorfenapyr) during the 2021 mass campaign. Four additional vector bionomics monitoring sites (for a total of eight) will be established in 2020 to gather additional data on the entomological impacts of the new net country-wide distribution.

LIBR will resume analysis of the sporozoite rate using ELISA in January. Once VectorLink trains LIBR staff on PCR techniques, LIBR staff will conduct additional molecular analysis.

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6. ANNEXES

ANNEX A: LOCATIONS OF INSECTICIDE RESISTANCE MONITORING SITES IN LIBERIA FROM NOVEMBER 2018 THROUGH SEPTEMBER 2019

County	Insecticide Resistance Testing Site (District)	Larval Collection Site for Insecticide Resistance (CDC Bottle Assay)	Status
Gbarpolu	Bopolu	Bopolu	November 2018
Lofa	Zorzor	Zorzor	February 2019
Grand Cape Mount	Sinje	Sinje	March 2019
Sinoe	Greenville	Greenville	May 2019
Grand Kru	Barclayville	Barclayville	June 2019
Grand Gedeh	Tchen District	Zwedru	July 2019
Nimba	Sanniquellee	Zolowee	August 2019
Bong	Jorquelleh District	Central Agricultural Research Institute (CARI)	September 2019

ANNEX B: NUMBER OF *AN. GAMBIAE* S.L. COLLECTED MONTHLY IN THE FOUR VECTOR BIONOMICS MONITORING SITES USING CDC-LTS INDOORS, FROM OCTOBER 2018 TO SEPTEMBER 2019.

Month	Fissebu	Koryah	Madina	Saint John	Total
October-18	0	7	1	5	13
November-18	0	10	0	2	12
December-18	0	2	0	0	2
January-19	0	5	2	1	8
February-19	0	17	0	0	17
March-19	0	4	0	0	4
April-19	8	30	3	17	58
May-19	8	10	1	36	55
June-19	2	9	1	18	30
July-19	9	2	1	13	25
August-19	2	3	0	3	8
September-19	2	3	0	3	8
Grand Total	**31**	**102**	**9**	**98**	**240**

ANNEX C: NUMBER OF *AN. GAMBIAE* S.L. COLLECTED MONTHLY IN THE FOUR VECTOR BIONOMICS MONITORING SITES USING CDC-LTS OUTDOORS, FROM OCTOBER 2018 TO SEPTEMBER 2019.

Month	Fissebu	Koryah	Madina	Saint John	Total
October-18	0	2	2	2	6
November-18	1	0	0	0	1
December-18	0	2	0	0	2
January-19	1	1	0	1	3
February-19	0	0	0	0	0
March-19	2	0	0	0	2
April-19	7	23	2	6	38
May-19	1	3	2	7	13
June-19	2	8	9	2	21
July-19	3	2	3	39	47
August-19	1	6	0	9	16
September-19	1	2	0	1	4
Total	**19**	**49**	**18**	**67**	**153**

ANNEX D: NUMBER OF *AN. FUNESTUS* S.L. COLLECTED MONTHLY IN THE FOUR VECTOR BIONOMICS MONITORING SITES USING CDC-LTS INDOORS, FROM OCTOBER 2018 TO SEPTEMBER 2019.

Month	Fissebu	Koryah	Madina	Saint John	Total
October-18	0	0	0	0	0
November-18	0	0	0	0	0
December-18	1	0	0	0	1
January-19	0	3	0	0	3
February-19	4	4	0	0	8
March-19	0	0	0	0	0
April-19	9	6	0	0	15
May-19	5	6	0	0	11
June-19	4	6	0	0	10
July-19	4	2	0	0	6
August-19	0	1	0	0	1
September-19	1	2	0	2	5
Total	**28**	**30**	**0**	**2**	**60**

ANNEX E: NUMBER OF *AN. FUNESTUS* S.L. COLLECTED MONTHLY IN THE FOUR VECTOR BIONOMICS MONITORING SITES USING CDC-LTS OUTDOORS, FROM OCTOBER 2018 TO SEPTEMBER 2019.

Outdoor	Fissebu	Koryah	Madina	Saint John	Grand Total
October-18	0	0	0	0	0
November-18	0	0	0	0	0
December-18	3	0	0	0	3
January-19	0	1	0	0	1
February-19	0	1	0	0	1
March-19	0	0	0	0	0
April-19	2	1	0	0	3
May-19	0	0	0	0	0
June-19	4	0	0	0	4
July-19	2	1	0	0	3
August-19	2	10	0	0	12
September-19	6	8	0	1	15
Grand Total	**19**	**22**	**0**	**1**	**42**